The histology and ultrastructure of the adductor muscle of the eastern oyster *Crassostrea virginica* (Gmelin)

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Abstract. The structure of the translucent and opaque parts of the adductor muscle of the eastern oyster *Crassostrea virginica* (Gmelin) was studied by light and transmission electron microscopy. The muscle was fixed with the valves held closed and after the muscle had relaxed so that the valves gaped. Several fixatives were used, some with recently developed modifications. The muscle fibres of the translucent and opaque parts of the adductor muscle have a similar organisation, with central myofilaments and peripheral sarcoplasmic reticulum, mitochondria and nucleus. In translucent muscle, the thick myofilaments are thinner than they are in opaque muscle, and the dense bodies are sometimes obliquely oriented. Nerve endings and invaginations of the sarcolemma at hemidesmosomes are shown for the first time in oyster adductor muscle. For the first time, presence of paramyosin is indicated by diagonal as well as transverse periodicities in sections of the thick myofilaments of obliquely striated muscle. These muscles are similar to the anterior byssus retractor muscle of the blue mussel *Mytilus edulis* Linné, except that no nexal junctions were found between the muscle fibres.

Larval oysters develop two adductor muscles, the "dimyarian" condition, but following attachment the anterior muscle degenerates (Galtsoff, 1964; Elston, 1980), resulting in the "monomyarian" condition. Adult bivalves which burrow retain two adductors, which are needed for the movements involved in burrowing; but two adductors are not necessary for the sessile oyster. The single adductor is large and more or less centrally placed, facilitating rapid closing in adverse conditions or when clearing the mantle of detritus. It is made up mainly of translucent muscle, which can contract and close the valves quickly. At one side of the adductor muscle is a smaller, crescent-shaped portion of opaque muscle that contracts more slowly, but can hold the valves shut against the tension of the hinge ligament for several days (Millman, 1964), a phenomenon known as "catch".

The gross structure (Galtsoff, 1964; Morrison and Odense, 1973), light microscopy and ultrastructure of the myofilaments and isolated paramyosin filaments of the adductor muscle of the eastern oyster *Crassostrea virginica* (Gmelin) have been described (Philpott *et al.*, 1960; Galtsoff, 1964; Morrison *et al.*, 1970; Cohen *et al.*, 1971; Morrison and Odense, 1974). The most detailed study of the adductor muscle of an oyster is that of the translucent part of the muscle of *Crassostrea angulata* (Lamarck) by Hanson and Lowy (1961). The relationship between thick and thin myofilaments and the structure of isolated thick myofilaments of *C. angulata* has also been studied (Lowy and Hanson, 1962; Elliott, 1964, 1974 and 1979; Hoyle, 1964; Elliott and Lowy, 1970). Bowden (1958) and Salánki and Zs-Nagy (1966) describe the appearance of the muscle fibres in *Ostrea edulis* L. using light

microscopy only. Bennett and Elliott (1981) and Elliott and Bennett (1982) show the myofilaments of *O. edulis* in sectioned material, but the other cell organelles were poorly fixed. They also show thick myofilaments isolated from the translucent and opaque parts of the adductor of *O. edulis*, and a striated appearance in tilted cross sections of thick myofilaments from the opaque part. Hanson and Lowy (1960) studied the myofilaments only of an oyster.

These studies were focused on the myofilaments because of the interest in the "catch" mechanism. Also, consistently good fixation of the other organelles was difficult to achieve. However, recently modifications of fixatives have been developed which seem to preserve tissues more completely. The present study was undertaken with the expectation that the ultrastructure of the myofilaments and the other organelles could be better preserved, to provide a more complete description of the oyster adductor muscle.

METHODS

Fixatives used were 4% glutaraldehyde in seawater (Morrison, 1970), Karnovsky's fixative (1% glutaraldehyde; 4% formalin prepared from paraformaldehyde, in phosphate buffer; Karnovsky, 1965), 1G4F (1% glutaraldehyde; 4% commercial formalin in phosphate buffer, McDowell, 1978), 1G4F with seawater replacing some of the distilled water (Howard and Smith, 1983), or 2.5% glutaraldehyde in 0.05 M caccodylate buffer (David Sims, pers. comm.). Generally, 1G4F with sea-water added gave better results than with phosphate buffer only, so only micrographs of muscle fixed with the

former fixative are shown. All solutions were used at pH 7.2.

To obtain fixed preparations of adductor muscles with the valves closed, part of the shell and the tissue around the adductor were removed to permit access of the fixative to the muscle, then the whole oyster was immersed in fixative. To obtain preparations of lengthened adductor muscle, the oyster was placed in 4°C seawater containing 8% $MgSO_4$ (Galtsoff, 1964) or 3.75% $MgCl_2$ (Hanson and Lowy, 1961), which cause the muscle to relax, until the valves gaped. The time taken for this varied from one to several hours, and the gape varied from 1-6 mm. The gape did not appear to alter when the oyster was touched, but to make sure the valves stayed apart dental wax was inserted between the valves. After removing part of the tissue surrounding the adductor muscle to expose the surface, the oyster was placed in fixative. After 1.5 to 2 hours, part of the exposed surface of the adductor muscle fixed at both lengths was removed and small pieces were placed in fresh fixative.

For light microscopy (LM) the specimens were dehydrated in methanol, then embedded in JB4 resin. For transmission electron microscopy (TEM) they were then fixed in 1% osmium tetroxide, dehydrated in acetone then embedded in Taab resin. JB4 resin sections were stained with a 1:50 dilution of 1% toluidine blue in 1% sodium borate, methylene blue/basic fuchsin, or Van Gieson stain (Dougherty, 1981). Taab resin semi-thin sections were also stained with toluidine blue for light microscopy. Sections for electron microscopy were stained with 25% uranyl acetate in methanol (Stempack and Ward, 1964), and lead citrate (Reynolds, 1963), and viewed in an Hitachi transmission electron microscope model MS-9.

Paramyosin paracrystals were prepared from the opaque and translucent part of the adductor, using the method described by Philpott *et al.* (1960) and Johnson *et al.* (1959), who worked on the muscles of several bivalves including *Crassostrea virginica.*

RESULTS

Better general fixation was obtained than in previous studies of oyster muscle. However, results were variable, often within the same section. Areas of poor fixation were sometimes found near the surface as well as deeper in the tissues. 1G4F with seawater was the most dependable fixative. There were periodicities in the thick myofilaments, and details of other cell organelles that were not evident in our earlier studies (Morrison, 1970; Morrison and Odense, 1974).

TRANSLUCENT MUSCLE

The translucent muscle consists of groups of elongate, thin muscle cells or fibres separated by a thin layer of connective tissue, the endomysium (Fig. 1). The elongate nuclei are oriented parallel to the long axis of the muscle cell. The

muscle fibres are ribbon-like, so in cross section they appear as flattened ovals about 3-4 μm wide and about 17 μm long, which sometimes bifurcate (Fig. 2). The fibres occur in groups, surrounded by a thicker layer of connective tissue, the perimysium.

Electron microscopy (Figs. 3, 4) reveals that each muscle fibre is composed of densely packed myofilaments and peripheral mitochondria, sarcoplasmic reticulum and a nucleus. The arrangement of thick and thin myofilaments and dense bodies is usually better shown in muscle which was fixed in an extended condition, after relaxation with $MgSO_4$ or $MgCl_2$ (Fig. 5) than in muscle from oysters fixed with the valves closed. The thin myofilaments are attached to isolated ''dense bodies'', which are oriented for short distances in oblique bands.

Typically, when viewed with light microscopy no striations are visible (Fig. 1). Oblique striations are occasionally seen, however, in fibres from preparations fixed with the valves closed where the anomalous effect known as ''supercontraction'' has occurred. The thick myofilaments pass into the regions at the ends of the sarcomeres where there are normally only dense bodies and thin myofilaments, often becoming folded so that obliquely aligned bands are produced, which are visible using light or electron microscopy (Figs. 6, 7).

In a cross section of a sample from an oyster relaxed with magnesium sulphate, fields of thick myofilaments surrounded by thin myofilaments, and of dense bodies among thin myofilaments, can be seen (Fig. 8). The thick myofilaments are regularly arranged in approximate hexagonal arrays.

At higher magnifications the thick myofilaments are cross-striated with a periodicity of about 5 nm. They also have a diagonal periodicity of about 35 nm and sometimes cross-links to adjacent thin filaments have a similar periodicity (Fig. 9). The transverse periodicity also occurs in isolated paracrystals of paramyosin, with every third bar accentuated (Fig. 10). Measurements of 100 thick myofilaments in cross section revealed a modal peak at about 32 nm (range = 18 to 62 nm).

Thin myofilaments are attached to the fusiform dense bodies (Fig. 11). The thick filaments are spindle-shaped, so they appear wider in the centre of a sarcomere and become smaller towards the dense bodies between sarcomeres (Fig. 12). As in mammalian skeletal muscle, where thick and thin filaments overlap the thick myofilaments are surrounded by a ring of thin filaments. This ring is sometimes incomplete around the wider thick myofilaments in the centre of the sarcomere, which would be equivalent to an H zone. Cross links can often be seen between adjacent thick and thin myofilaments and between adjacent thin myofilaments. Some of the dense bodies are attached to the sarcolemma, forming hemidesmosomes (Fig. 13). Filaments from the connective
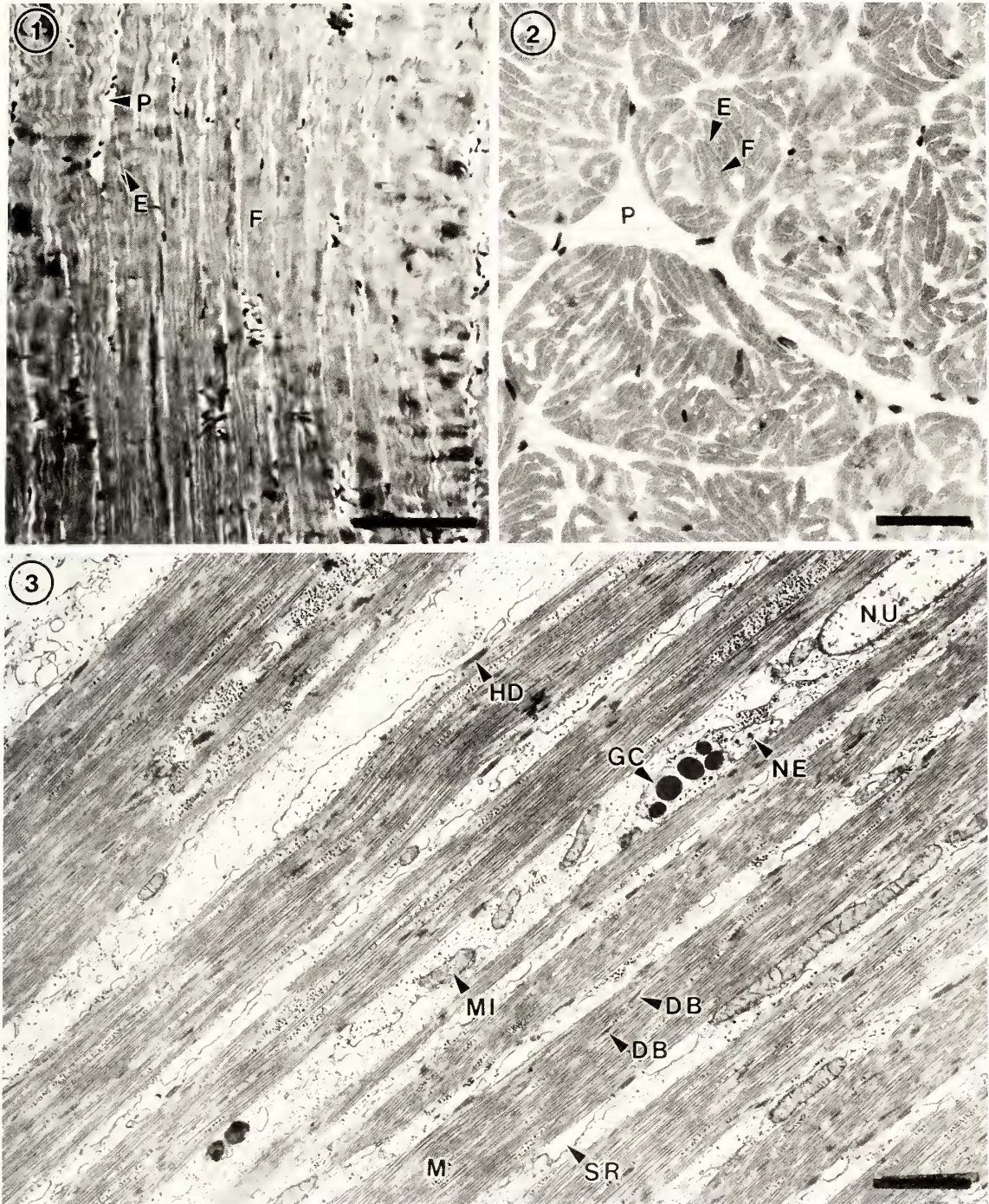
Fig. 1. Longitudinal section (parallel to the muscle fibres of the adductor extending between the two valves of the oyster) of translucent part of adductor muscle. Specimen was fixed in 2.5% glutaraldehyde in caccodylate buffer, embedded in JB4, and stained with toluidine blue. Long, thin muscle fibres (F) are surrounded by endomysium (E), and groups of muscle fibres are surrounded by perimysium (P) (scale bar = 0.1 mm). **Fig. 2.** Cross section (across the adductor muscle) of translucent part of adductor muscle. Specimen was fixed in 2.5% glutaraldehyde in caccodylate buffer, embedded in JB4, and stained with Van Gieson. The profiles of the muscle fibres (F) are elongate and sometimes bifurcate; each fibre is surrounded by endomysium (E), and groups of fibres are surrounded by perimysium (P) (scale bar = 30 μm). **Fig. 3.** Longitudinal section of translucent part of adductor muscle. TEM micrograph of specimen fixed in 1G4F. The muscle fibres have a central core of myofilaments (M) and dense bodies (DB), and hemidesmosomes (HD) are present at the sarcolemma. The peripheral cytoplasm contains elongate mitochondria (MI), sarcoplasmic reticulum (SR) and an elongate nucleus (NU). Between the muscle fibres are glial cells (GC) closely associated with nerve endings (NE) (scale bar = 2 μm).
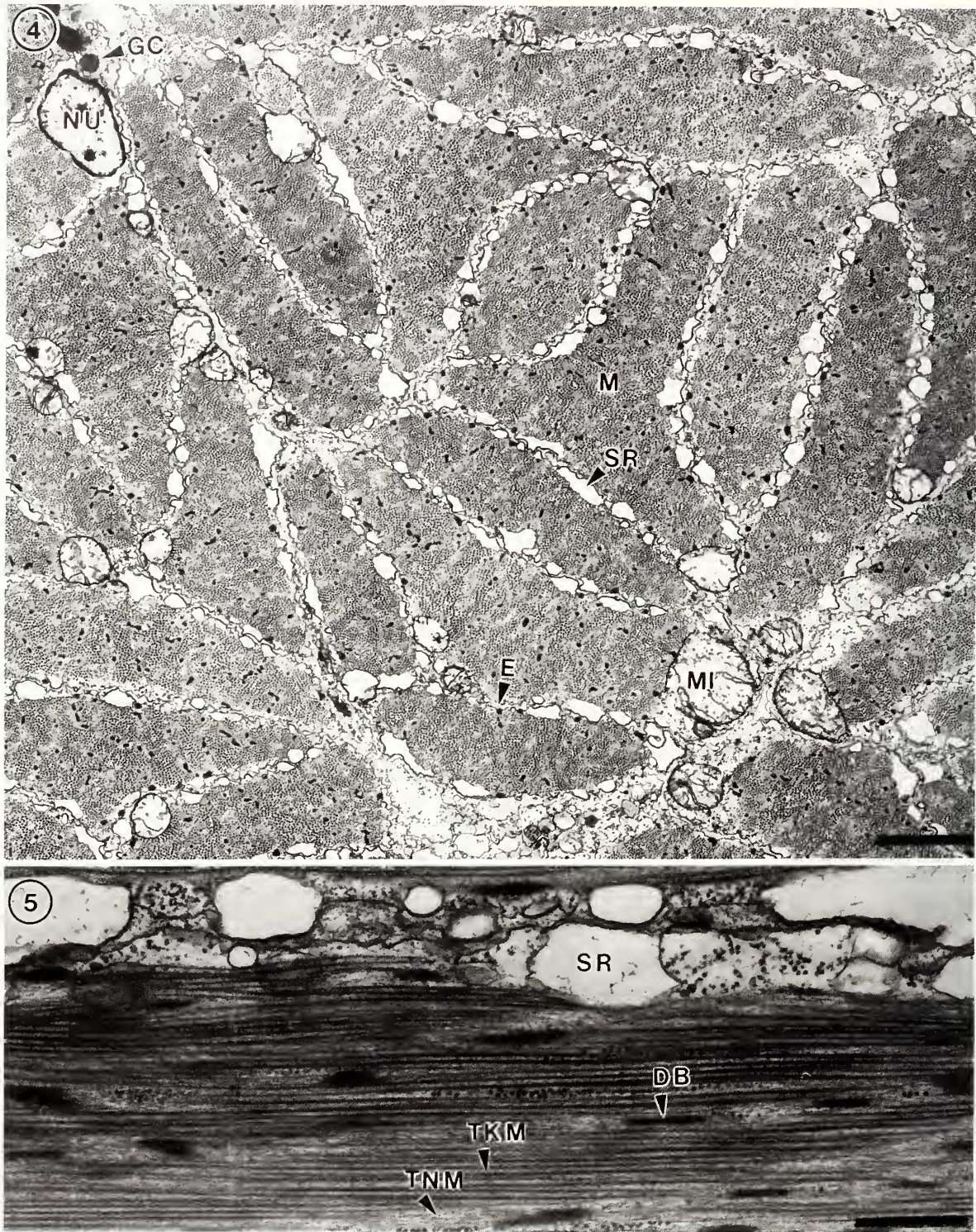
Fig. 4. TEM micrograph of translucent part of adductor muscle of specimen relaxed in Mg SO₄ and fixed in Karnovsky's fixative. The same features can be recognised as in figure 3, but the nucleus (NU), mitochondria (ME) and sarcoplasmic reticulum (SR) are more rounded since this is a cross section [(E) endomysium; (GC) glial cell; (M) myofilaments] (scale bar = 2 μm) **Fig. 5.** TEM micrograph of longitudinal section of muscle fibres of translucent part of adductor muscle. Specimen relaxed in Mg SO₄ and fixed in Karnovsky's fixative. Thick myofilaments (TKM) are present and thin myofilaments (TNM) are continuous with dense bodies (DB), which are obliquely oriented for short distances. There are profiles of sarcoplasmic reticulum (SR) beneath the sarcolemma (scale bar = 1 μm).
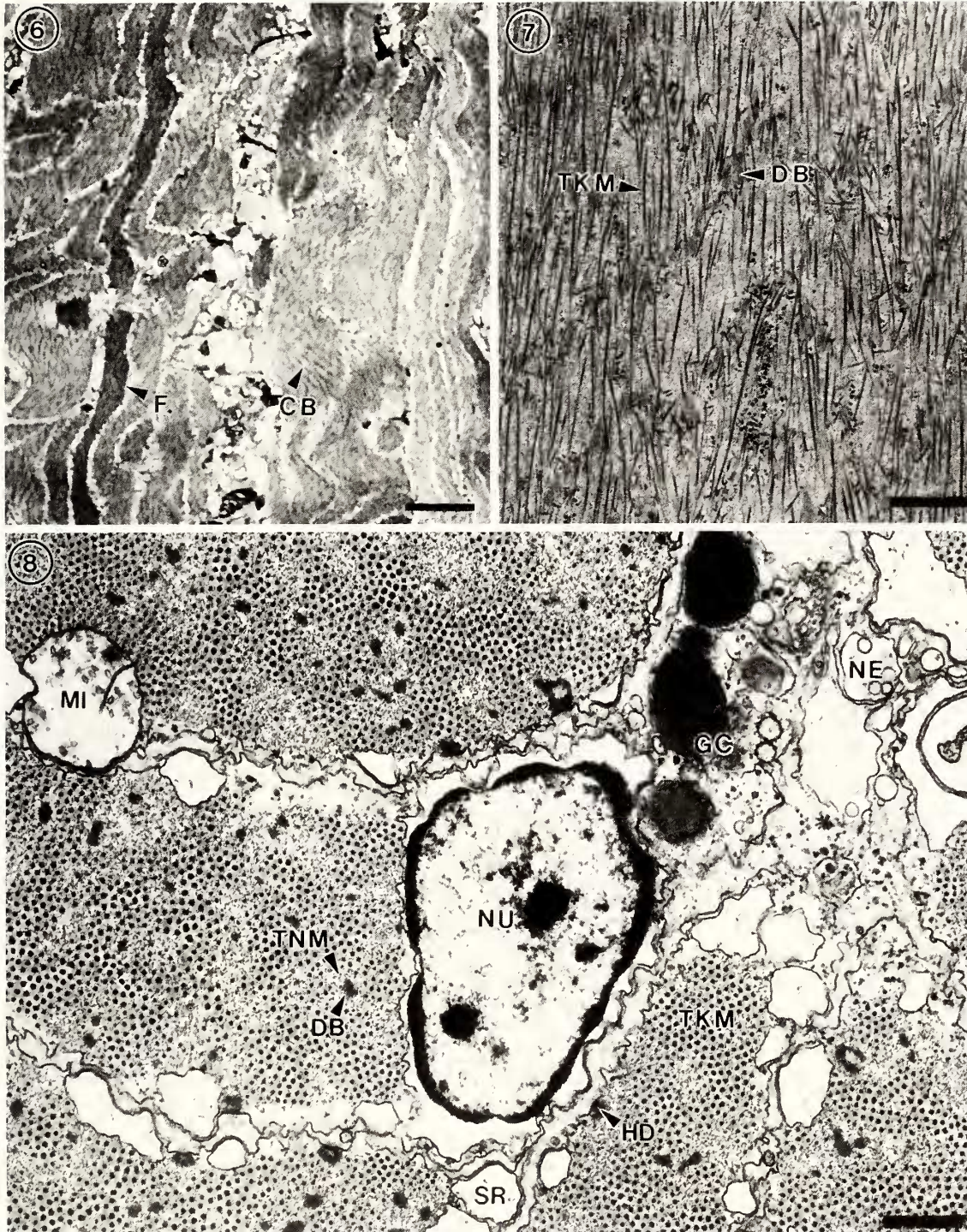
Fig. 6. Longitudinal section of translucent part of contracted adductor muscle of specimen fixed in 2.5% glutaraldehyde in caccodylate buffer and embedded for electron microscopy, 0.5 μm section stained with toluidine blue. Note oblique contraction bands (CB) in the fibres (F) (scale bar = 20 μm).
Fig. 7. TEM micrograph of longitudinal section of contraction bands of translucent part of contracted adductor muscle. Specimen fixed in 2.5% glutaraldehyde in caccodylate buffer. Thick myofilaments (TKM) overlap and become folded in regions where there are dense bodies (DB) (scale bar = 1 μm). **Fig. 8.** TEM micrograph of cross section of translucent part of adductor muscle. Specimen relaxed in Mg SO_4 and fixed in Karnovsky's fixative. There are fields of thick and thin myofilaments (TKM) and thin myofilaments (TNM) in the muscle fibres, and dense bodies (DB) occur in the fields of thin myofilaments. Hemidesmosomes (HD) present at the sarcolemma. The nucleus (NU) is rounded in cross-section. Vesicles of sarcoplasmic reticulum (SR) as well as mitochondria (MI) occur beneath the sarcolemma. A glial cell (GC) with an accompanying nerve ending (NE) is present between the muscle cells (scale bar = 500 nm).

tissue stroma accumulate next to the hemidesmosome. The sarcolemma is often invaginated where there are hemidesmosomes (Figs. 3, 8).

The profiles of sarcoplasmic reticulum are situated just beneath the sarcolemma, and dense material can be seen between the outer membrane of the sarcoplasmic reticulum and the sarcolemma (Fig. 14). The sarcolemmas of adjacent muscle cells are often close to each other (Figs. 3-5, 8), but no well-defined junctions were seen. Many of the mitochondria in one specimen have filamentous paracrystals and annulated cristae (Fig. 15).

Nerve endings, which contain a variety of vesicles, some small and clear, some dense-cored or of varying density, often occur close to the sarcolemma (Figs. 3, 16). Glial cells containing large granules, the gliosomes, are usually closely associated with these nerve endings but do not completely surround them.

OPAQUE MUSCLE

The muscle fibres of the opaque muscle are more rounded in cross section than those of translucent muscle, so their diameter (10-20 μm) is similar to that of the length but larger than the width of the oval profiles of the translucent muscle fibres (Figs. 17, 18). In the transitional zone between the translucent and opaque muscle the muscle fibres of each type are intermingled (Fig. 19). As in the translucent muscle, each muscle fibre is surrounded by endomysium, and the groups of fibres by perimysium. The arrangement of cell organelles is also similar. Central myofilaments are surrounded by vesicles of sarcoplasmic reticulum and mitochondria, and the sarcolemmas of neighbouring cells are often closely opposed (Figs. 20, 21). The nucleus is situated in cytoplasm to one side of the cell, oriented with its long axis parallel with that of the muscle cell, and dense material can be seen between the sarcolemma and the sarcoplasmic reticulum (Fig. 22).

The thick myofilaments are thicker than those of the translucent muscle, having a modal peak at about 61 nm (range = 18 to 123 nm). They exhibit cross striations at 5 nm and often diagonal striations like those of translucent muscle, but they are more evident (Figs. 23, 24). Every third cross striation is accentuated. Cross links occur between the thick and thin myofilaments (Figs. 25, 26), and some thin myofilaments have cross links to more than one thick myofilament (Fig. 25). As in the thick myofilaments of the translucent muscle fibres, the cross links seem to have a similar periodicity to that of the diagonal bands.

Sometimes, there is a single ring of thin myofilaments surrounding each thick myofilament in the region of overlap, but more often there are several thin myofilaments between the thick myofilaments. Cross links can sometimes be seen between adjacent thin myofilaments, and occasionally connections can also be seen between thick myofilaments

(Fig. 26). When the thick myofilaments are cut obliquely, they often appear to be banded (Fig. 27).

The thin filaments are attached to dense bodies (Fig. 28) which do not show any special arrangement in the muscle fibre (Fig. 29), so this type of muscle is classified as smooth. As in the translucent muscle fibre, some dense bodies are attached to the sarcolemma, forming hemidesmosomes (Fig. 30), and filaments of connective tissue in the endomysium are closely associated with the sarcolemma at these sites. In some places the sarcoplasm surrounding the myofilaments is wide, and sarcolemmal invaginations containing filaments of connective tissue extend to the hemidesmosomes (Fig. 31). Glial cells and axons occur between the muscle fibres. Nerve endings, often accompanied by glial cells, are found next to the sarcolemma, sometimes embedded in the sarcoplasm (Fig. 32). They contain a variety of small clear and dense-cored vesicles and larger vesicles containing a varying amount of dense material.

DISCUSSION

The adductor muscle of oysters, as in most bivalved molluscs, consists of uninucleate muscle cells of small diameter (3-20 μm) that are much longer than those of vertebrate smooth muscle (Twarog, 1976). Each muscle cell contain a single myofibril, and peripheral vesicles of sarcoplasmic reticulum which form couplings with the sarcolemma, as described in the adductor muscle of the scallop, *Argopecten irradians* (Lamarck, 1819) (Nunzi and Franzini-Armstrong, 1981) and the translucent part of the adductor muscle of *Crassostrea angulata* (Hanson and Lowy, 1961). The invaginations of the sarcolemma at the hemidesmosomes are not as well defined as the transverse tubular system of transversely striated muscle or some unstriated muscle (Dorsett and Roberts, 1980), but may perform a similar transport function.

The dense bodies, like the Z-line of striated muscles, anchor the actin filaments. In the translucent part of the adductor of *Crassostrea virginica* they form oblique bands as in *C. angulata*, in which the bands are arranged helically around the outer part of the fibre and branch and anastomose in the centre of the muscle fibre (Hanson and Lowy, 1961). These bands were not seen in normal muscle in this study, but can be seen using phase contrast illumination (Hanson and Lowy, 1961). The contraction bands of supercontracted muscle are obvious using regular as well as phase illumination (Bowden, 1958; Hanson and Lowy, 1961; Galtsoff, 1964; Salánki and Zs-Nagy, 1966; Morrison and Odense, 1974). More regular oblique banding occurs in some molluscs such as the octopus and cuttlefish, where the myofibrils surround a central core of mitochondria (Millman, 1967). In this study, the dense bodies, and the actin filaments entering them, were easier to see in specimens fixed in 4% glutaraldehyde in sea
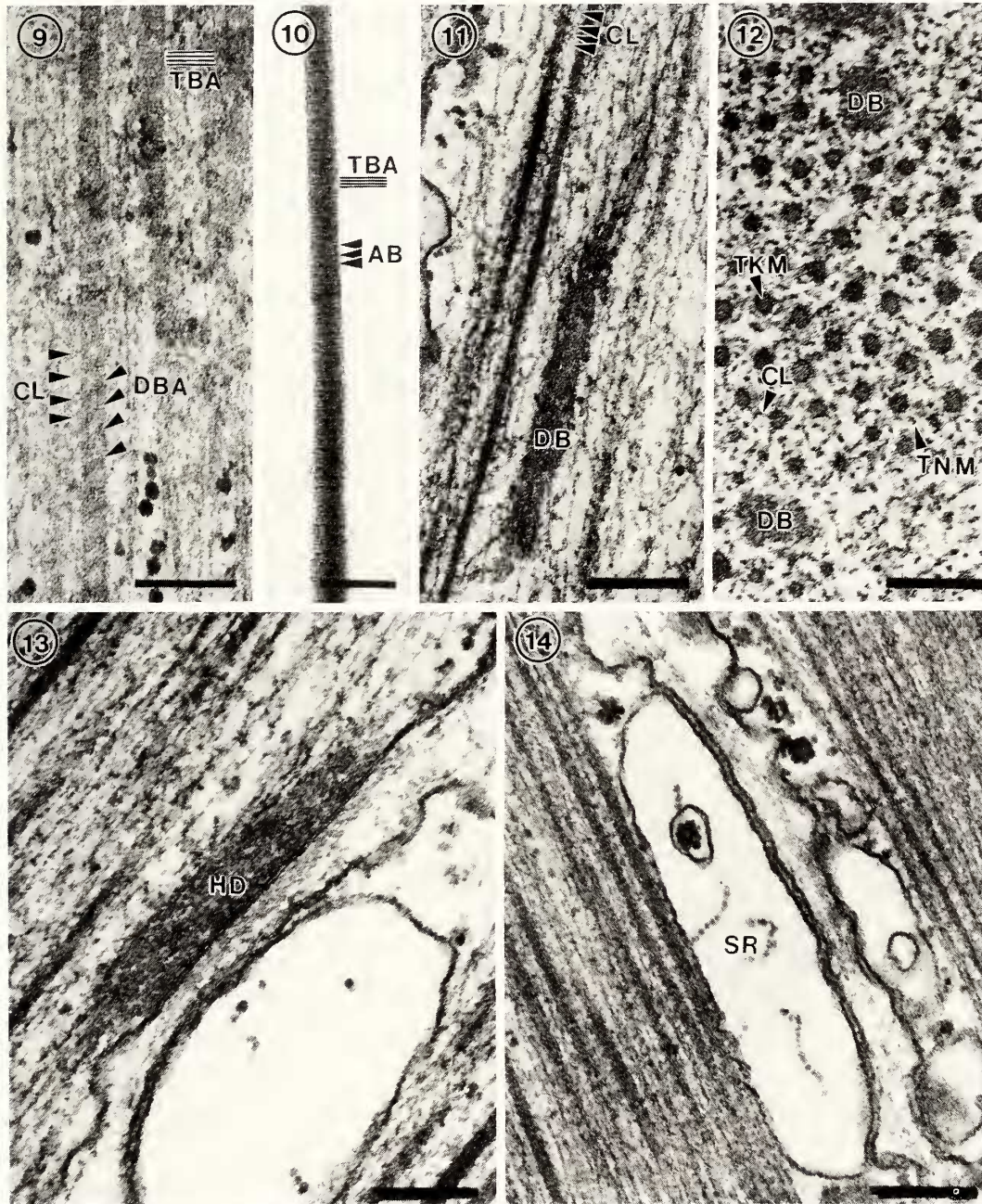
Fig. 9. TEM micrograph of longitudinal section of translucent part of adductor muscle of specimen fixed in 2.5% glutaraldehyde in caccodylate buffer. The thick myofilaments exhibit transverse banding (TBA) at intervals of about 15 nm and diagonal banding (DBA). Cross-links (CL) to the actin filaments can be seen in some areas, at about the same intervals as the diagonal banding (scale bar = 100 nm). Fig. 10. Translucent part of adductor muscle showing paramyosin paracrystal with narrow transverse bands (TBA) about 5 nm apart and accentuated bands (AB) about 15 nm apart (scale bar = 10 nm). Fig. 11. TEM micrograph of longitudinal section of translucent part of adductor muscle of specimen relaxed in Mg SO$_4$ and fixed in Karnovsky's fixative. Cross links (CL) occur between the thick and thin myofilaments, as well as between the thin filaments. The latter enter dense bodies (DB) (scale bar = 200 nm). Fig. 12. TEM micrograph of cross section of translucent part of adductor muscle of specimen relaxed in Mg SO$_4$ and fixed in Karnovsky's fixative. Thick myofilaments (TKM), thin myofilaments (TNM) and dense bodies (DB) are present. Each thick myofilament is surrounded by a ring of thin myofilaments, and cross-links (CL) can often be seen between the two types of myofilament (scale bar = 100 nm). Fig. 13. TEM micrograph of longitudinal section of translucent part of adductor muscle of specimen relaxed in Mg SO$_4$ and fixed in Karnovsky's fixative. There is a hemidesmosome (HD) to one side of the double membrane forming the sarcolemma; on the other side there are connective tissue filaments (scale bar = 200 nm). Fig. 14. TEM micrograph of longitudinal section of translucent part of adductor muscle of specimen fixed in iG4F. A thin layer of dense material is present between a vesicle of sarcoplasmic reticulum (SR) and the sarcolemma (scale bar = 200 nm).
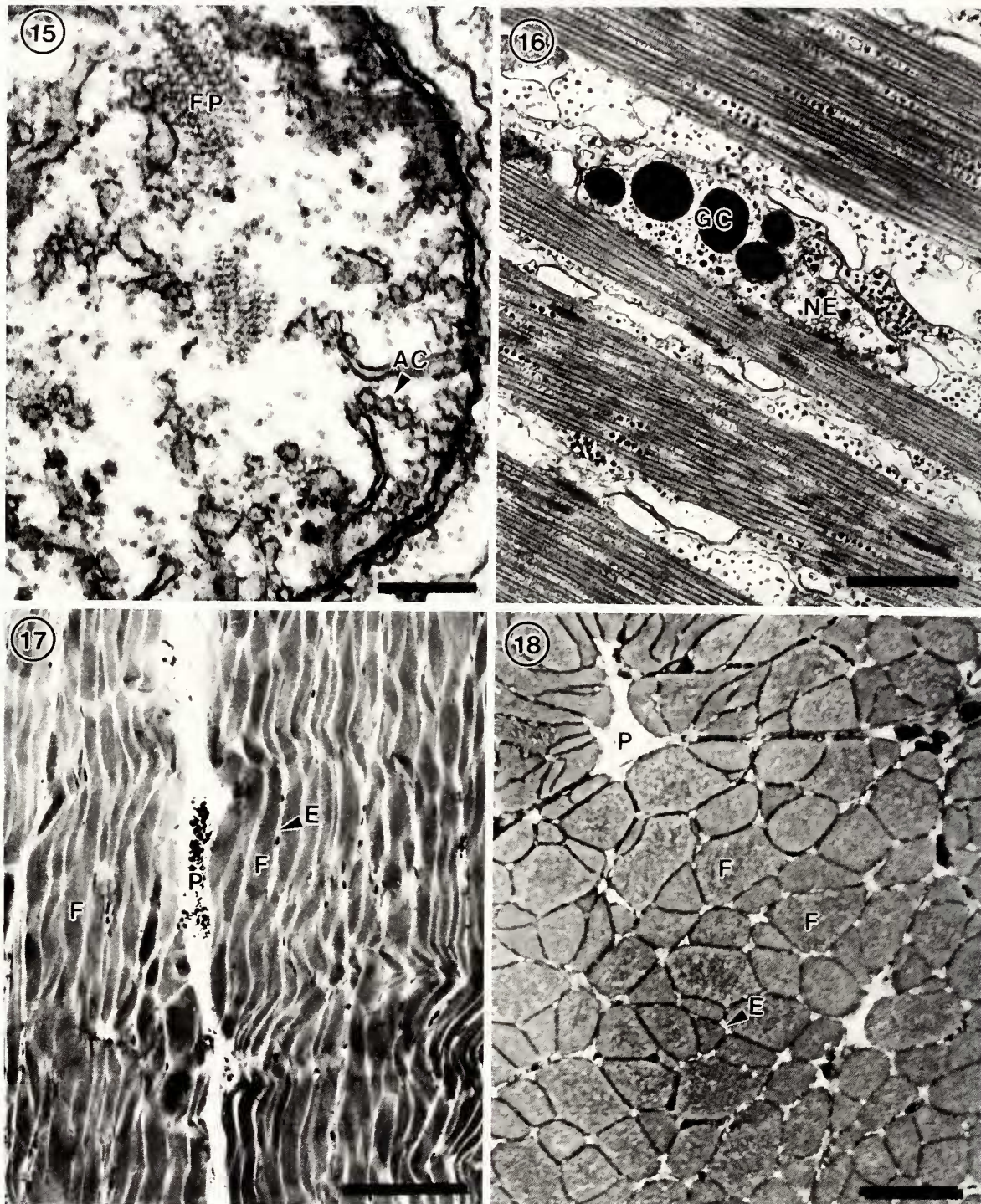
Fig. 15. TEM micrograph of transverse section of translucent part of adductor muscle of specimen relaxed in Mg SO$_4$ and fixed in Karnovsky's fixative. A mitochondrion contains filamentous paracrystals (FP) and annulated cristae (AC) (scale bar = 200 nm). **Fig. 16.** TEM micrograph of longitudinal section of translucent part of adductor muscle of specimen fixed in 1G4F. There is a nerve-ending (NE) containing clear and dense vesicles, and an associated glial cell (GC) near the sarcolemma (scale bar = 1 µm). **Fig. 17.** Longitudinal section of opaque part of adductor muscle of specimen fixed in 2.5% glutaraldehyde in caccodylate buffer, embedded in JB4 and stained with toluidine blue. The muscle fibres (F) that are wider than those of the translucent muscle, and are separated by endomysium (E) and perimysium (P) (scale bar = 0.1 mm). **Fig 18.** Cross section of opaque part of adductor muscle of specimen fixed in 2.5% glutaraldehyde in caccodylate buffer, embedded in JB4 and stained with methylene blue/basic fuschin. The muscle fibres (F) are large and have a rounded profile [(E) endomysium; (P) perimysium] (scale bar = 20 µm).

water used in earlier work than in more recently developed fixatives, presumably because less background material in the cell was fixed.

Dense bodies also form hemidesmosomes at the sarcolemma in the anterior byssus retractor muscle (ABRM), where it has been suggested that they give structural stability to the muscle by attaching the muscle cells to the closely associated filaments of connective tissue (Twarog, 1967; Twarog et al., 1973) and propagate tension to neighboring cells via the collagen of the connective tissue (Sobieszek, 1973; Twarog, 1976). Hanson and Lowy (1961) suggested, in their study on the translucent adductor muscle of Crassostrea angulata, that the sarcolemma may be interrupted next to the dense body, but it can be clearly seen that this is not the case in the present study (Fig. 13).

In the ABRM nexal or gap junctions about 15 nm across which may be sites of intercellular conductivity have been described between the muscle cells (Twarog et al., 1973), but these were not seen in the present study. However, the sarcolemmas of adjacent cells often run parallel to each other farther apart, but over greater distances than those found in nexal junctions. This has been reported in several other molluscs (Zs.-Nagy and Salánki, 1970; Nicaise and Amsellem, 1983).

The filamentous paracrystals found in the mitochondria of one specimen are similar to those described in the mitochondria of the myocardial cells of Crassostrea virginica (Hawkins et al., 1980). These workers also found prismatic cristae, and our specimen had annulated cristae. Their specimens, like ours, appeared to be otherwise normal, although in other animals paracrystals are usually associated with disease or states of altered metabolism.

Glial cells like those found in both the translucent and opaque parts of the adductor muscle in this study have been described forming a "glio-interstitial network" between tonic muscle cells in a variety of molluscs, including the tonic muscle of the adductor of Anodonta, and the ABRM of Mytilus edulis (Amsellem et al., 1973; Gilloteaux, 1975; Nicaise and Amsellem, 1983). They have not previously been recorded in phasic muscles. These authors suggest that these cells may help to control metabolic exchanges between the interstitial spaces and the nerves and/or muscles.

The opaque muscle tissue of the adductor examined in this study is very similar in appearance and physiology to the ABRM of the mussel (Lowy and Hanson, 1962; Millman, 1964; Twarog, 1967; Heumann and Zebe, 1968; and Sobieszek, 1973). However, its thick myofilaments are wider than those of the ABRM, which have a peak width of about 40 nm (Sobieszek, 1973). Unlike the translucent muscle, there is no obvious arrangement of dense bodies. Lowy and Hanson (1962) assert that some degree of order is necessary for the sliding-filament mechanism to work efficiently, and such an order has been demonstrated in the

ABRM using optical diffraction techniques (Sobieszek, 1973). Sobieszek postulates that there are sarcomeres consisting of three to four thick myofilaments which are 25 μm long, thin myofilaments which are 140 μm long and two dense body halves. Such sarcomeres would be difficult to recognise in sectioned material, since they are so long and narrow, so they may be present in the opaque adductor muscle of Crassostrea virginica. Cross-bridges between the thick and thin myofilaments have been shown in other mollusc muscle cells, such as those of the ABRM, in varying degrees of contraction. It has been suggested that direct interaction occurs between the thick myofilaments (Hoyle, 1983), as indicated by the close relationship sometimes seen between them (Fig. 26); but other workers believe that this does not play a part in contraction (Bennett and Elliot, 1989).

The thick myofilaments of vertebrate striated muscle are very constant in diameter (about 16 nm) and length (about 1.6 μm); whereas the thick myofilaments of most mollusc fibres, including those described in the present study, are wider and vary in width (Levine et al., 1976). This is associated with the presence of varying amounts of paramyosin (Millman, 1967). The proportion of paramyosin in transversely striated muscles such as the fast part of the adductor of Placopecten magellanicus (Gmelin) is low (7% by mass; Chantler, 1991), but is higher in obliquely striated muscle such as that of the translucent part of the adductor of Crassostrea angulata (16-20%; Chantler, 1983) and highest in smooth muscle such as that of the opaque part of the adductor of C. angulata (22-39%; Chantler, 1983). Paramyosin forms the core of the thick myofilament, surrounded by myosin (Szent-Györgyi et al., 1971) and gives a typical X-ray diffraction pattern originally described by Bear (1944) and Bear and Selby (1956), which subsequently became known as the Bear-Selby net. The transverse striations with a periodicity of about 15 nm, and diagonal striations with a periodicity of about 35 nm described in the thick myofilaments of the translucent and opaque muscle in this study form a "checkerboard" pattern characteristic of the paramyosin core of "catch" muscle (Hanson and Lowy, 1964; Chantler, 1991). The diagonal striations have not been shown previously in a translucent or phasic muscle. These periodicities were first shown by Hall et al. (1945) to correspond to the pattern formed by the Bear-Selby net, and result from the tendency of paramyosin molecules to assemble with a 14.5 nm intermolecular shift as a result of intermolecular ionic interactions (Kendrick-Jones et al., 1969; Castellani and Cohen, 1987). The relationship between the patterns seen in paramyosin and myofilament preparations from "catch" muscle using the electron microscope and the Bear-Selby net are considered in detail by Elliott and Lowy (1970), Cohen et al. (1971), and Elliott (1979). The banding patterns seen in thick myofilaments in oblique sections of the opaque muscle in the present study (Fig. 27) have also been
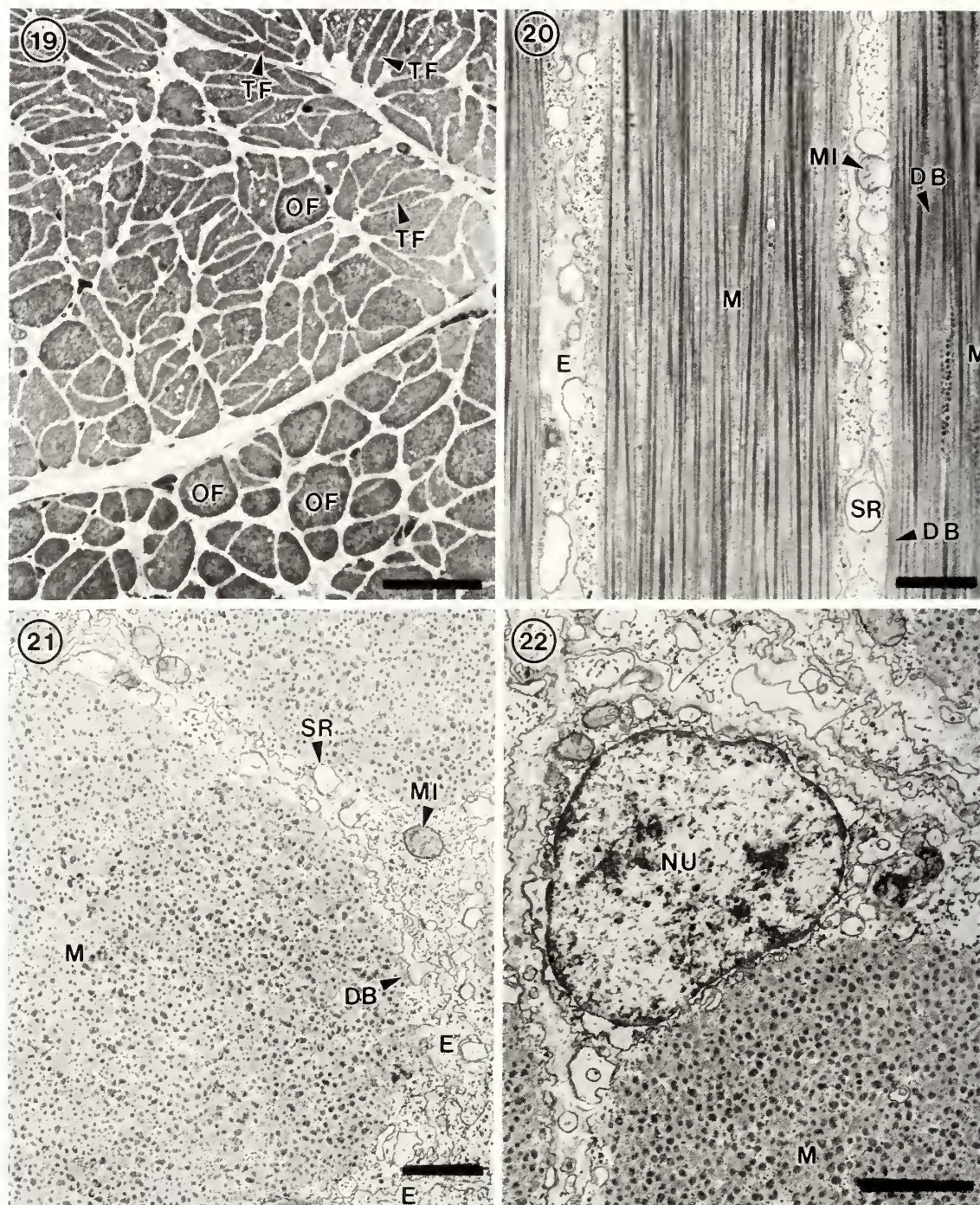
Fig. 19. Transverse section of the transition zone between the opaque and translucent parts of the adductor muscle of specimen fixed in 2.5% glutaraldehyde in caccodylate buffer, embedded in JB4 and stained with methylene blue-basic fuschin. Thick opaque fibres (OF) can be seen to the bottom of the micrograph, and more elongate, thinner translucent ones (TF) can be seen to the top. In the centre fibres of both types are intermingled (scale bar = 20 μm). **Fig. 20.** TEM micrograph of longitudinal section of opaque part of adductor muscle of specimen relaxed in Mg Cl_2 and fixed in 2.5% glutaraldehyde in caccodylate buffer. A central core of myofilaments (M) and dense bodies (DB) is present in each muscle fibre. There are mitochondria (MI) and vesicles of sarcoplasmic reticulum (SR) next to the sarcolemma. These are not as elongate as in translucent muscle. Endomysium (E) is present between muscle fibres (scale bar = 1 μm). **Fig. 21.** TEM micrograph of cross section of opaque part of adductor muscle of specimen fixed in Karnovsky's fixative, showing the dense bodies (D), endomysium (E), myofilaments (M), mitochondria (MI) and sarcoplasmic reticulum (SR) seen in longitudinal section in figure 20 (scale bar = 1 μm). **Fig. 22.** TEM micrograph of cross section of opaque part of adductor muscle fixed in Karnovsky's fixative. The nucleus (NU) appears rounded, and is in the cytoplasm to one side of the myofilaments (M) (scale bar = 1 μm).
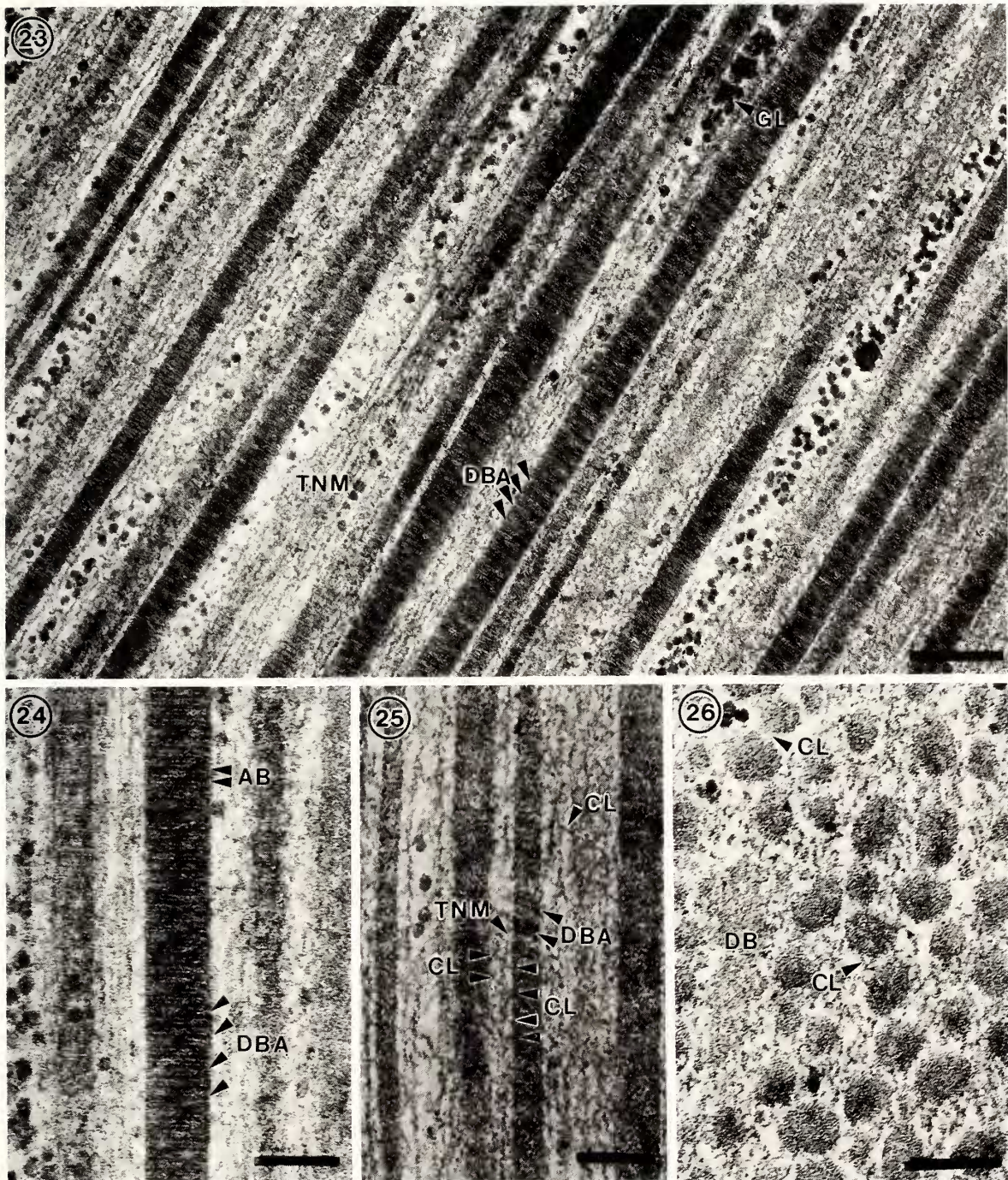
Fig. 23. TEM micrograph of longitudinal section of opaque part of adductor muscle of specimen fixed in 2.5% glutaraidehyde in caccodylate buffer. The thick myofilaments have transverse and diagonal banding (DBA), and sometimes seem to be very close together. Thin filaments (TNM) and glycogen (GL) can also be seen (scale bar = 200 nm). **Fig. 24.** TEM micrograph of longitudinal section of opaque part of adductor muscle of specimen fixed in 2.5% glutaraldehyde in caccodylate buffer. Transverse banding can be seen, with every third band accentuated (AB). Diagonal banding (DBA) is also present (scale bar = 100 nm). **Fig. 25.** TEM micrograph of longitudinal section of opaque part of adductor muscle of specimen fixed in Karnovsky's fixative. There are cross-links (CL) between thick and thin myofilaments, and also between thin myofilaments. CL have a similar periodicity to the diagonal bands (DBA) on the thick myofilaments. One thin myofilament (TNM) has links to two thick myofilaments (scale bar = 100 nm). **Fig. 26.** TEM micrograph of cross section of opaque part of adductor muscle of specimen relaxed in Mg Cl₂ and fixed in 2.5% glutaraldehyde in caccodylate buffer. Cross-links (CL) are present between thick and thin myofilaments, and also occasionally between thick myofilaments. A dense body (DB) can be distinguished (scale bar = 100 nm).
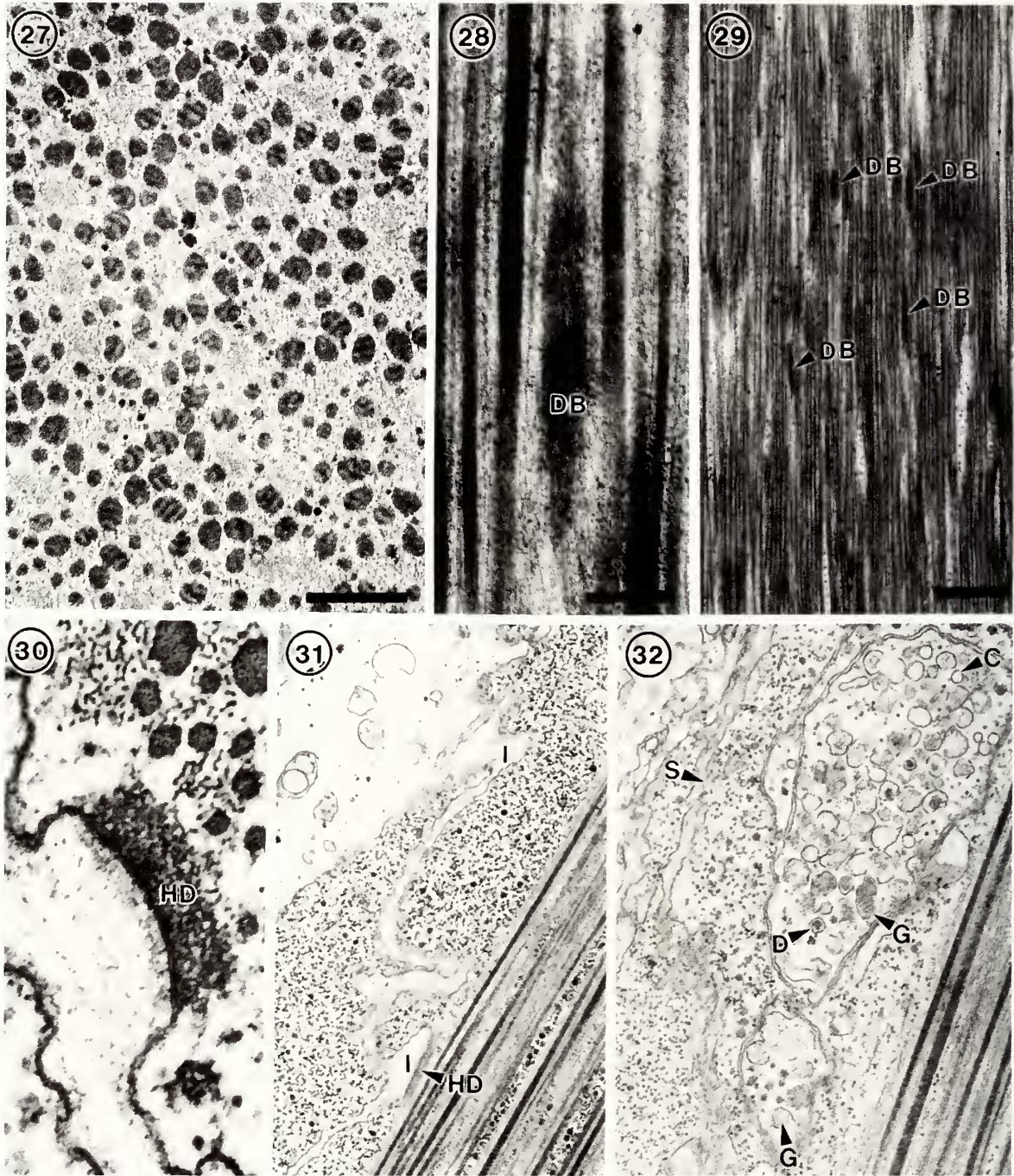
Fig. 27. TEM micrograph of cross section of opaque part of adductor muscle of specimen relaxed in Mg Cl₂ and fixed in 2.5% glutaraldehyde in caccodylate buffer. Light and dark bands cross the thick myofilaments (scale bar = 300 nm). **Fig. 28.** TEM micrograph of longitudinal section of opaque part of adductor muscle fixed in 4% glutaraldehyde in seawater. Thin actin myofilaments enter the dense body (DB) (scale bar = 300 nm). **Fig. 29.** TEM micrograph of longitudinal section of opaque part of adductor muscle of specimen fixed in 4% glutaraldehyde in seawater. There is no definite pattern in the distribution of the dense bodies (DB) (scale bar = 1 μm). **Fig. 30.** TEM micrograph of cross section of opaque part of adductor muscle of specimen relaxed in Mg SO₄ and fixed in Karnovsky's fixative. The sarcolemma is invaginated next to a hemidesmosome (HD), and connective tissue filaments are concentrated in the adjacent endomysium (scale bar = l00 nm). **Fig. 31.** TEM micrograph of longitudinal section of opaque part of adductor muscle of specimen fixed in Karnovsky's fixative. A long invagination of the sarcolemma extends to a hemidesmosome (HD) at the sarcolemma (scale bar = 1 μm). **Fig. 32.** TEM micrograph of longitudinal section of opaque part of adductor muscle of specimen fixed in 2.5% glutaraldehyde in caccodylate buffer. Nerve endings are embedded in the sarcoplasm and contain small clear (C) and dense-cored vesicles (D) as well as vesicles with varying amounts of granular contents (G), which are usually larger than the other types. An invagination (I) of the sarcolemma (S) extends to the myofibril (scale bar = 500 nm).

shown in *Ostrea edulis, C. angulata, C. gigas* Thunberg, 1793, *Mercenaria mercenaria* (Linné, 1758), *Pecten maximus* (Linné, 1758) and the ABRM of *Mytilus edulis* when cross sectioned material was tilted (Bennett and Elliott, 1981; Elliott and Bennett, 1982), and these authors show that these patterns are caused by viewing the myofilaments down planes of the Bear-Selby net.

The thin myofilaments of the sea scallop adductor muscle have been shown to be very similar to those in vertebrates (Chantler, 1991). The thin myofilaments of the oyster have a similar appearance and diameter, suggesting that they also have a similar composition. In the translucent adductor muscle, there appear to be about 12 thin myofilaments around each thick myofilament, as reported in *Crassostrea angulata* (Hanson and Lowy, 1961).

The muscle fibres of the opaque part of the adductor of *Placopecten magellanicus* (Cohen *et al.*, 1971) sometimes have obliquely aligned dense bodies, and the thick myofilaments have a similar peak diameter, 36 nm (Morrison and Odense, 1974) as those of the obliquely striated, translucent muscle of the oyster. They contain paramyosin and exhibit a small degree of "catch" (Hoyle, 1964). The obliquely striated muscle of the oyster also has some ability to maintain "catch" (Hanson and Lowy, 1961), and its X-ray pattern shows the Bear-Selby net typical of paramyosin (Elliott and Bennett, 1982). The presence of paramyosin is also demonstrated by the transverse and diagonal periodicities found in sectioned material in the present study. These two types of muscle are therefore very similar, although one forms the opaque, slow part and the other the fast, translucent part of an adductor muscle.

Smooth and obliquely striated muscles in the adductors of bivalves have the same arrangement of organelles, and in this study their thick myofilaments show the same banding patterns. There appears to be a spectrum of muscle cell types in bivalve adductor muscles, with slightly varying ultrastructural characteristics, the latter producing a graded series of physiological characteristics.

ACKNOWLEDGMENTS

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